Reproductive Biology of *Colus stimpsoni* - III

(Prosobranchia : Buccinidae)

Female Genital System [1]

BY

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(1 Plate; 6 Text figures)

INTRODUCTION

The order Neogastropoda is considered to contain the most advanced prosobranchs, and all its members have internal fertilization (Hyman, 1967; Fretter & Graham, 1962). These gastropods deposit their eggs within a resistant egg capsule which is attached in clusters or singly to various substrata. Generally, neogastropods deposit many eggs within an individual capsule; whereas, in the majority of other prosobranchs each egg is deposited in an encasing shell, along with its supply of nutrient albumin. Also, many neogastropods, in which the development has been studied, exhibit suppression of a free living larval stage, and the young emerge as miniature adults. In those species which exhibit direct development, many hundreds of eggs are deposited within a single capsule, but only a few develop. The remaining undeveloped eggs ("nutritive eggs" or "nurse eggs") serve as food for the young (Thorson, 1935, 1940; Radwin & Chamberlin, 1973; Moore & Sander, 1978; Lyons & Spight, 1973).

With the habits of depositing numerous eggs within a capsule and of internal fertilization, the female genital system has evolved and specialized in accordance with these behaviors (Fretter, 1941, 1946, 1953). Since fertilization must occur before nutritive and capsule forming materials are secreted around the eggs, spermatozoa must be transferred or deposited into the region of the oviduct preceding the secretory areas. However, spermatozoa are generally deposited at the terminal end of the female duct. Spermatozoa may be stored at the terminal end, within the bursa copulatrix, or they may be passed up the oviduct and stored within specialized regions connected to the gonoduct, such as the seminal receptacle or the ingesting gland (Fretter, 1941, 1953; Houston, 1976).

Organization of the female neogastropod genital system appears to be rather uniform throughout the order (Ponder, 1974; Houston, 1976; Smith, 1967; Fretter, 1941, 1946), with differences in the location of the seminal receptacle, ingesting gland and bursa copulatrix, or in the presence or absence of some of these structures. The female system generally consists of a single tube extending from the ovary, along the visceral mass, into the mantle cavity where it passes along the roof. In lower gastropods, the ovary is connected to the right nephridium, and the gametes are discharged into the water via the nephridiopore. However, in neogastropods the ovary opens into the mantle region by a duct. Various terms, some indicating a functional relation, others having phylogenetic implications, have been applied to the duct extending along the visceral mass. However, in the present study, the term "renal oviduct" will be used to denote that portion of the oviduct extending along the visceral mass, and "pallial oviduct" for that portion extending along the mantle roof. The genital ducts of the muricid *Thais (Nucella) lapillus* (Linnaeus, 1758) and the buccinid *Buccinum undatum* Linnaeus, 1758, are frequently used as examples of neogastropod reproductive systems and are considered typical of this order (Hyman, 1967; Fretter & Graham, 1962; Ponder, 1974). However, many members of the Buccinidae have not been investigated. The present study concerns the female reproductive system of the buccinid *Colus stimpsoni* (Mörch, 1867) to further the knowledge of its phylogenetic relationships and its relation to the reproductive strategy of direct development and nutritive eggs.

MATERIALS AND METHODS

Snails of various sizes were collected intertidally at Cobscook State Park, Edmunds, Maine, and at Eastport, Maine, and maintained in running sea water aquaria (West, 1973, 1978). To determine the sex of individual

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snails, the animals were placed on a dry table with the body aperture facing upward. After a few minutes the snails would try to right themselves. During this period, the presence or absence of a penis could be noted as the foot extended over the edge of the shell. Snails were segregated according to sex, measured, and an identifying number affixed to the shell.

For histological studies, tissues were excised from freshly opened snails and processed according to the methods described in the first paper of this series (West, 1978).

RESULTS

General Morphology

The ovary, a deep orange-brown to orange-yellow in color, lies on the distal-most portion of the visceral mass in the ultimate and in part of the penultimate whorls of the shell (Figure 1). The ovary and visceral mass are covered by a single layer of cuboidal epithelium, the pallial epithelium, and a thin layer (20 - 60 μm thick) of connective tissue and muscle. In large females (80 mm or greater in shell length), the ovary may cover more than one-half of the digestive gland.

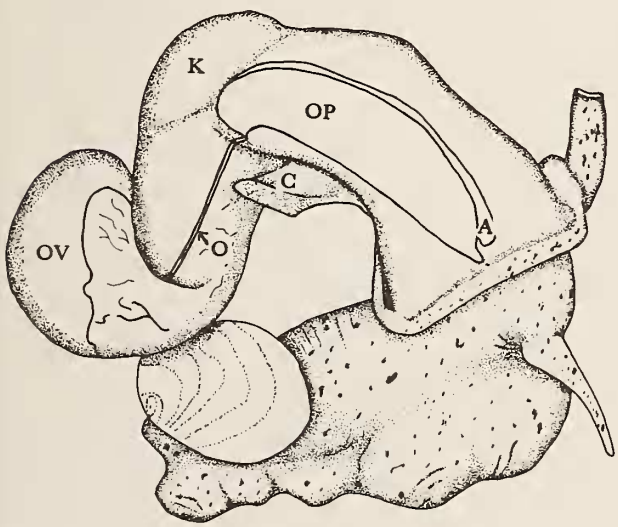


Figure 1

Female *Colus stimpsoni*: whole animal with shell removed and the mantle drawn as transparent: A – anus; C – columellar muscle; D – digestive gland; K – kidney; OV – ovary; OP – pallial oviduct; O – renal oviduct

The oviduct emerges from the ovary and passes as a single straight duct along the columellar side of the visceral mass beneath the pallial epithelium. At the posterior limits of the mantle cavity, the oviduct turns abruptly dorsad and enlarges. The enlarged portion reflexes anteriorly and continues in the mantle roof, alongside the rectum. The oviduct consists of 2 morphologically distinct portions, a thin-walled renal oviduct which passes along the visceral mass, and a glandular, pallial oviduct which passes along the mantle (Figure 1). The pallial oviduct is opaque white in color and is the most noticeable portion of the genital duct in gross dissection. The pallial oviduct narrows at its anterior end forming a short vagina. In sexually mature females, the pallial oviduct varies from 3 to 6 cm in length and is oval in cross section, measuring 5 to 18 mm in long axis and 3 to 9 mm in short axis.

Near the junction of the renal and pallial oviducts, at the posterior end of the mantle cavity, a small duct, the gonopericardial duct, opens into the renal oviduct. The gonopericardial duct (Figure 2) passes posteroventrally toward the pericardium. However, this duct could only be traced to within a very short distance from the pericardium.

Histology

Ovary: The ovary is a multitubular organ with the tubules generally oriented perpendicular to the spiral axis of the shell. The ovary is separated from the digestive gland, but ovarian tubes occasionally intrude between the tubules of the digestive gland. Ovarian tubules are separated from one another by a layer (2 - 8 μm in thickness) of loose connective tissue and muscle fibers. Beneath this layer is a basal lamina which varies from 0.1 - 0.3 μm in thickness and is composed of a dense layer of fibers. Young oocytes and follicle cells lie on the periphery of the tubule subjacent to the basal lamina. Vitellogenic and postvitellogenic phases of oocyte development occur in the center of the tubule. The ovarian tubules eventually join to form the single oviduct.

Renal Oviduct: The thin-walled renal oviduct is embedded in loose connective tissue and varies from 500 to 800 μm in diameter (Figure 3). The wall (10 to 20 μm thick) is composed of circular muscle and connective tissue. The duct is lined with a simple columnar epithelium which rests on an indistinct basal lamina. Epithelial cells vary in height with tall cuneiform cells projecting into the lumen at irregular intervals. These tall cells (75 - 100 μm in height) are surrounded by decreasingly shorter cells

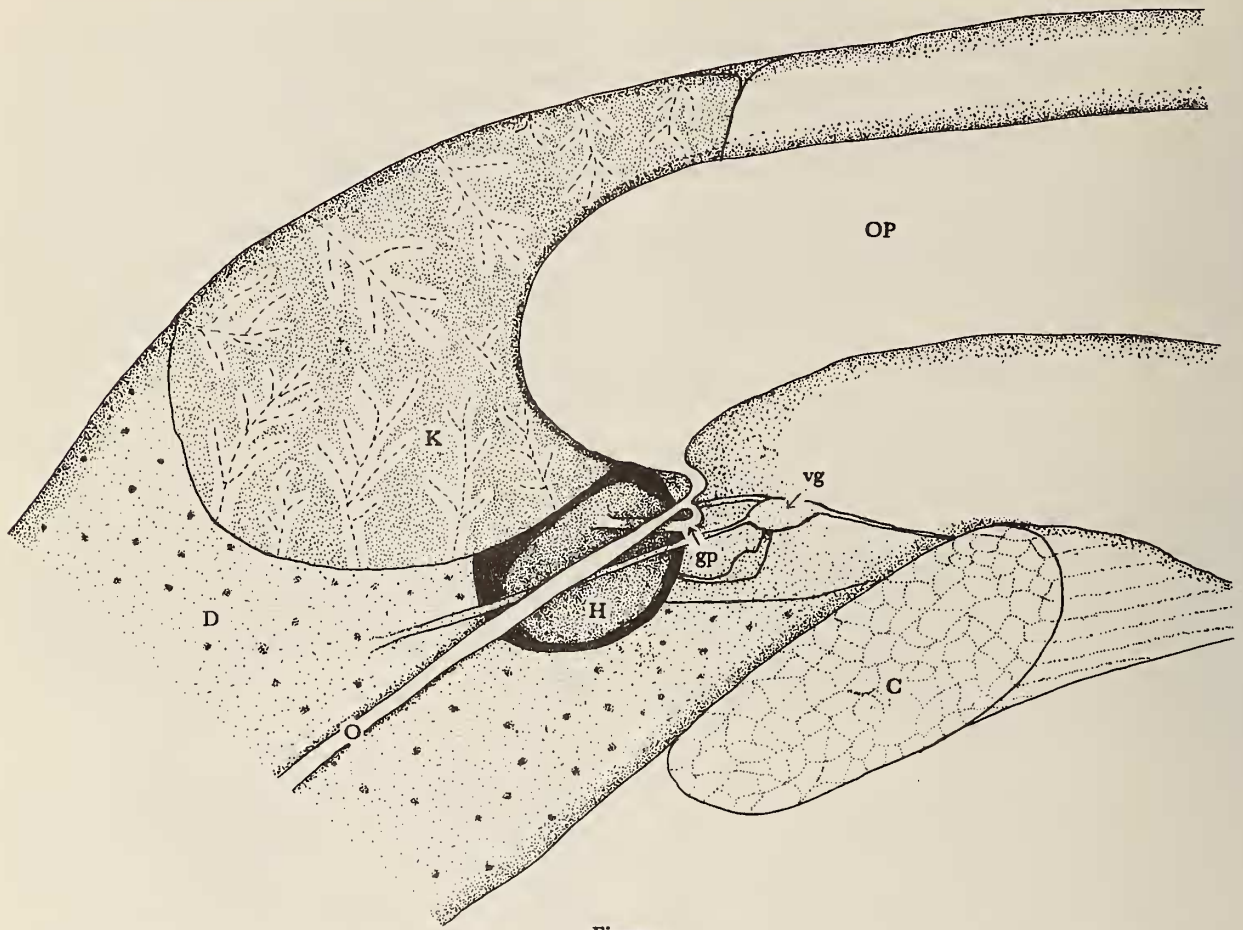


Figure 2

Drawing of gonopericardial duct and its relation to the genital duct: C – columellar muscle; D – digestive gland; GP – gonopericardial duct; H – heart; K – kidney; OP – pallial oviduct; O – renal oviduct; VG – visceral ganglion. (Not drawn to scale)

with the shortest cells measuring 25 - 30 μm in height. The variation in cell height gives the epithelium a mucosa-like appearance. Nuclei of the epithelial cells are irregular in outline and vary from 7 - 12 μm in length. Occasionally, within the renal oviduct, disrupted oocytes are observed, and the surrounding epithelial cells contain yolk platelets.

Pallial Oviduct: The renal oviduct turns sharply dorsad near the posterior limits of the mantle cavity and becomes glandular, forming the pallial oviduct. The pallial oviduct is composed of 3 parts: a glandular region, a bursa copulatrix, and the vagina. These components of the oviduct are surrounded by a layer of connective tissue and muscle giving the appearance of a single enlarged tube.

Explanation of Figures 6 to 8, 10 and 11

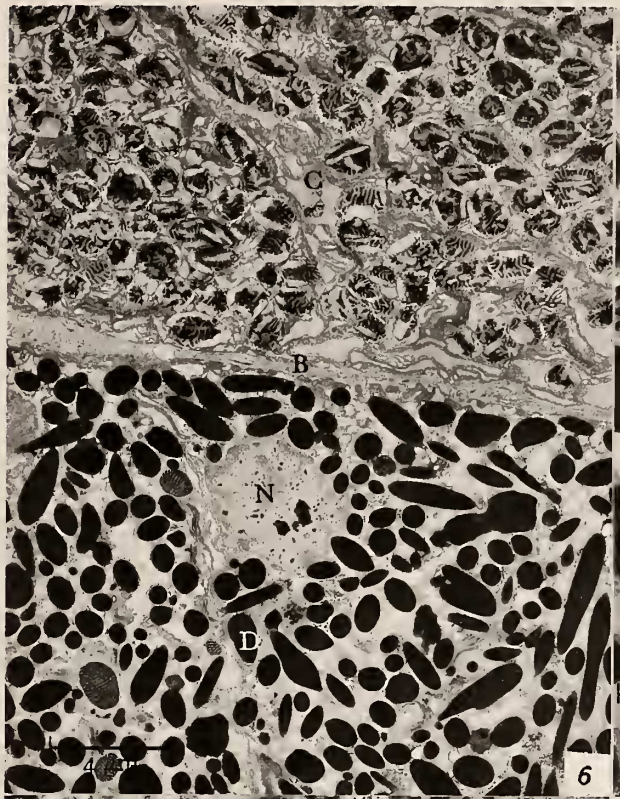
Figure 6: Electron micrograph of capsule gland wall showing the dorsal differential-staining region (D) and the central region (C): B – basal lamina and connective tissue between tubules; N – nucleus

Figure 7: Electron micrograph of gland cell granules within dorsal and ventral differential-staining regions of the capsule gland

Figure 8: Electron micrograph of the gland cell granules within the central region of the capsule gland

Figure 10: Egg capsule of *Colus stimpsoni*: L – larva; O – capsule operculum

Figure 11: Light micrograph of egg capsule wall: C – central layer; I – inner layer; O – outer layer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6


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7


1 µm
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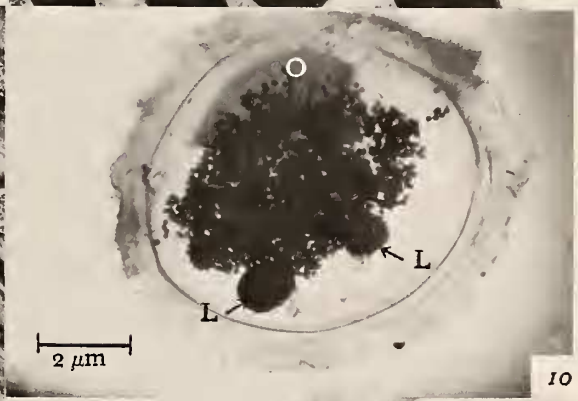
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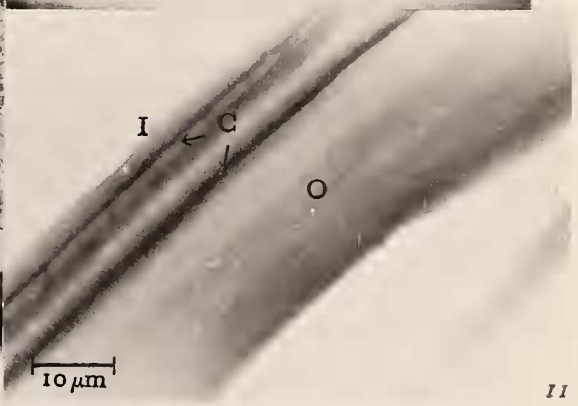
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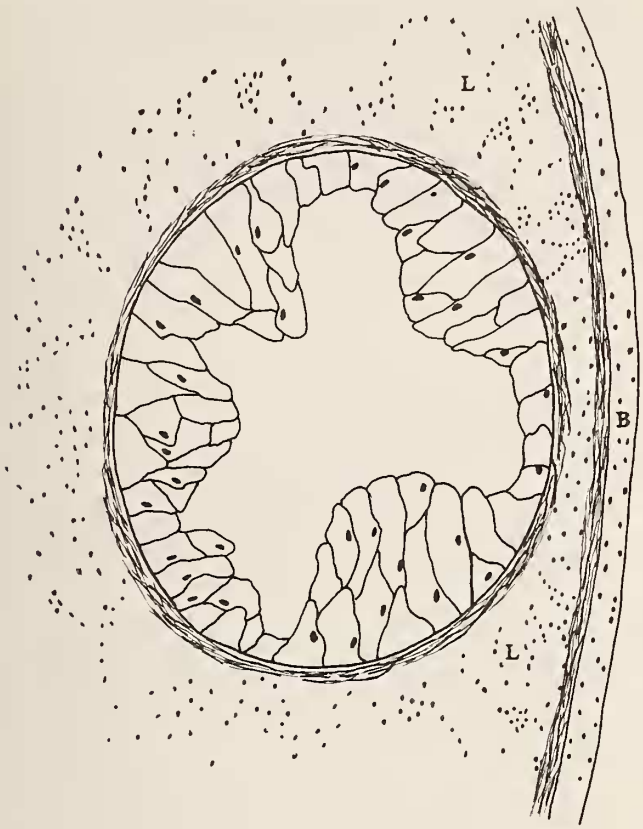

Figure 3

Line drawing of a cross section through renal oviduct: B – body wall; L – loose connective tissue

Slightly posterior to the transition region between the pallial and visceral portions, the oviduct reflexes sharply posteriad and passes out of the mantle into the body between the kidney and body wall. This portion is parallel and dorsal to the renal oviduct. At a point about $\frac{1}{3}$ along the kidney, the oviduct turns abruptly forward and passes back into the mantle. These turns form an S-shaped loop. This loop is covered by the connective tissue surrounding the pallial oviduct and is not visible in gross dissections, as shown in Figure 2.

The glandular segment constitutes the largest part of the pallial oviduct and is histologically similar along its length but has differentially staining regions. The posterior end (5 - 10 mm in length) corresponds to the albumin gland reported in other neogastropods and the remaining glandular area corresponds to the capsule gland (Fretter, 1941; Houston, 1976). In cross section, the albumin and capsule glands are composed of right and left lobes. These lobes are connected dorsally and ventrally by relatively thin walls which give the lumen of the oviduct the appearance of a dorso-ventral slit (Figure 4).

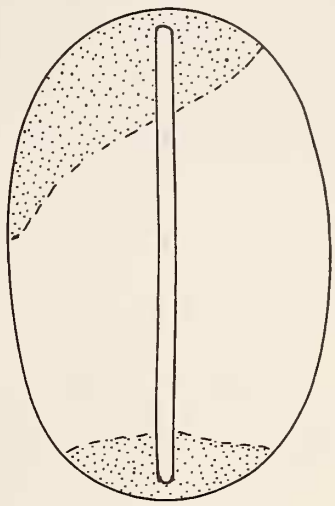

Figure 4

Line drawing of a cross section through the capsule gland; stippled areas show extent of dorsal and ventral differential-staining regions

The epithelium of the pallial oviduct consists of tall, ciliated columnar cells (20 - 40 μm in height), which have elongated, ellipsoidal nuclei, and numerous gland cells (Figure 5). The gland cells are tightly packed together, forming rod-shaped or tubular glands which are elongated and coiled distally. These glands are packed together, and each is enclosed by the basal lamina of the epithelium (Figure 6). Gland cells are large with subspherical, basally located nuclei. These cells are elongated, and the necks of the cells extend to the surface through the center of the gland (Figure 5). The glands have no lumen *per se*, but rather a core of cell necks which are filled with secretory granules. While the distal cells of a gland have much longer necks than do the proximal ones, there is no apparent difference in the diameter. Glands along the dorsal and ventral walls of the oviduct are shorter in length (0.3 - 0.5 mm vs. 1.5 mm) than ones along the lateral walls (see Figure 4). Blood lacunae are irregularly distributed throughout the walls of the pallial oviduct and

appear as numerous, small spaces in sectioned material (Figure 5).

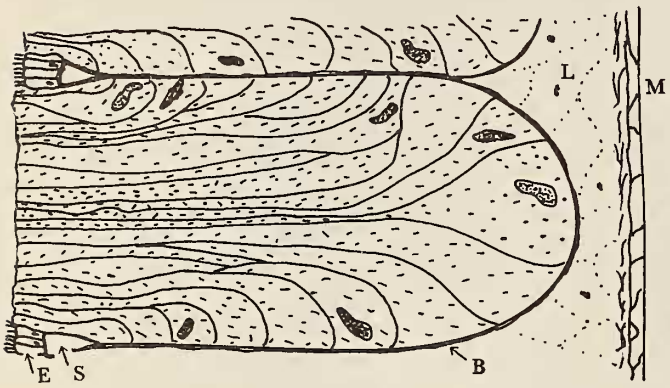

Figure 5

Line drawing of capsule gland wall showing tubule glands: B – basal lamina; M – mantle cavity; E – ciliated cells of epithelium; L – loose connective tissue; S – blood sinus. (Not drawn to scale)

The albumin and capsule glands stain differentially from one another with the azo-carmine procedure. The granules of the albumin gland cells stain predominantly pale blue; whereas, those of the capsule gland cells stain predominantly purple and pale violet. The albumin gland gives rise to the capsule gland at a region opposite the posterior end of the ctenidium. Near this area, the right dorsolateral wall of the albumin gland forms a fold of glandular tissue. This fold extends ventrally into the lumen and is attached to the ventral wall at the apex of the fold. This fold is about 5 mm in length and is covered laterally with a ciliated epithelium. The fold extends into the posterior limits of the capsule gland and divides the lumen into right and left portions.

The capsule gland walls are thicker than the albumin gland walls and have differentially staining areas within them. In cross section, the dorsal $\frac{1}{3}$ and ventral $\frac{1}{3}$ stain predominantly red to purple and the middle section stains predominantly blue to pale violet with the azo-carmine stain (Figure 4). The middle section also stains deep blue-black with Heidenhain's hematoxylin. Within these regions of the capsule gland, the gland cells contain both red and blue staining granules. However, these regions are dominated by one staining type of granule. The granules of the gland cells in the dorsal and ventral portions are predominantly fusiform to rod-shaped (Figure 7); whereas, the granules of the central portion are spherical (Figure 8).

The cytoplasm of the cells of the dorsal and ventral portions of the capsule gland is dominated by numerous vacuoles which contain flocculent material, in addition to the dense granules (Figure 7). The granules are electron-dense with alternating bands of slightly less electron densities, having a periodicity of about 250 Å (Figure 7). The cytoplasm contains a few mitochondria as well as glycogen particles and the Golgi complex. The cisternae of the Golgi complex are filled with an electron-dense material.

The granules of the gland cells within the central portion of the capsule gland are membrane-bounded and are composed of electron-dense granular material and a somewhat less electron-dense fibrous substance (Figure 8). The granular material is distributed in patches broken up by the fibrous material. Well-developed Golgi lamellae are scattered within the cytoplasm, and the cisternae contain an electron-dense granular substance. The cisternae of the rough endoplasmic reticulum are enlarged and contain dispersed granules which are about the same electron-opacity as the neighboring cytoplasm (Figure 8).

The bursa copulatrix is situated at the anterior end of the capsule gland (Figure 9). It is oval in lateral view, and the capsule gland slopes ventrally beneath it. The lumen of the oviduct passes beneath the bursa copulatrix, opening into the vagina, and the ciliated columnar epithelium (20 - 50 μm thick) of the oviduct is folded in this region. The bursa copulatrix is a muscular chamber and is separated from the capsule gland and the wall of the oviduct beneath it by a layer of loose connective tissue and muscle. The wall of the bursa measures 200 - 500 μm in thickness, and the epithelium is ciliated and folded. The duct connecting the bursa copulatrix and vagina is histologically similar to the bursa and is separated from the wall of the oviduct by a layer of loose connective tissue. Sperm fill the bursa copulatrix and its duct throughout the year. Around the periphery of the bursa, the heads of the sperm are oriented toward the epithelium and are in contact with it. The duct of the bursa copulatrix opens into the vagina a short distance from the female opening. The vagina is lined with columnar and mucous-secreting cells. Beneath this epithelium is a layer of muscle and connective tissue. The vagina is 5 - 10 mm in length and closed by a sphincter (Figure 9).

Cytochemistry of Pallial Oviduct

Results obtained from sections embedded in polyester wax and stained with Lehmann's polychrome indicate a num-